

strikingly similar to those that have been described for lead encephalopathy in children (5) and idiopathic amaurotic epilepsy of nonhuman primates (6-8). It is interesting to note that other workers have suspected that a relationship may exist between lead poisoning and amaurotic epilepsy (8, 9) of monkeys.

The last case described differs from the others in that sudden paraplegia was present rather than epilepsy; vascular lesions were minimum, and bilateral symmetrical demyelination was much more extensive. These signs and lesions were essentially the same as those described for idiopathic leukoencephalomyelosis of nonhuman primates, a disease that has occurred concurrently with amaurotic epilepsy (7).

The lesions associated with lead poisoning in these four primates suggest a new animal model for the study of demyelination and tend to support the contention of others that lead may in some way be a factor in certain idiopathic demyelinating diseases of animals (9, 10) and man (11).

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the concentration of melatonin (7) and norepinephrine (8, 9) in the pineal.

Male Osborne-Mendel rats (NIH strain) weighing 180 to 220 g were used. The intensity of light in cages was 108 to 340 lumen/m². Animals were killed by a blow to the head, and within a minute the pineals were removed. Glands were stored for less than 5 minutes at room temperature in Ringier injection solution while the extraneous tissue was removed. The activity of *N*-acetyltransferase was determined by a modification of a previous method (3). A single pineal gland was homogenized in 20 μ l of 0.1M sodium phosphate buffer (pH 6.8) containing [¹⁴C]serotonin (0.5 mM) and acetyl coenzyme A (0.5 mM). The reaction mixture was incubated for 10 minutes at 37°C. The [¹⁴C]*N*-acetylserotonin and [¹⁴C]melatonin that were formed during incubation were isolated by thin-layer chromatography and eluted, and the radioactivity was then determined.

A circadian rhythm in the activity of *N*-acetyltransferase is present in the rat pineal gland (Fig. 1). A 15-fold increase in enzyme activity occurs during the first 3 hours of the dark period, suggesting that darkness may cue the rhythm.

To determine whether this circadian rhythm could be endogenously generated in the absence of lighting shifts, we maintained some animals in continual darkness for 6 days. A comparison of the enzyme activity at 11:00 a.m. and 11:00 p.m. indicates that a rhythm does persist (Table 1). In the group tested at 11:00 a.m., the average of six of the seven enzyme activities was 45 units (a unit of activity is the number of picomoles of [¹⁴C]serotonin *N*-acetylated per gland homogenate per hour). The remaining pineal had an activity of 720 units. A similar variation in the distribution of enzyme activities was observed in the group tested at 11:00 p.m., with the majority of the values greater than 800 units. The greatly increased variability in the data indicates that the pineal *N*-acetyltransferase rhythm becomes asynchronous among a group of rats maintained in darkness.

The exposure of rats to continual lighting suppresses the *N*-acetyltransferase rhythm (Table 1). The measurement of *N*-acetyltransferase at six evenly spaced times during a 24-hour period provided no evidence that a rhythm was present (10). The sup-

Indole Metabolism in the Pineal Gland:

A Circadian Rhythm in *N*-Acetyltransferase

Abstract. *The activity of N-acetyltransferase in the rat pineal gland is more than 15 times higher at night than during the day. This circadian rhythm persists in complete darkness, or in blinded animals, and is suppressed in constant lighting. The N-acetyltransferase rhythm is 180° out of phase with the serotonin rhythm and is similar to the norepinephrine and melatonin rhythms. Experiments in vitro indicate that norepinephrine, not serotonin, regulates the activity of N-acetyltransferase through a highly specific receptor.*

N-Acetyltransferase converts serotonin to *N*-acetylserotonin (1). In the pineal gland, *N*-acetylserotonin is *O*-methylated by hydroxyindole-*O*-methyltransferase to form the pineal specific compound melatonin (2). Our studies with cultured rat pineal glands have indicated that *N*-acetyltransferase activity is stimulated manifold by the neurotransmitter norepinephrine by way of an adenylyl cyclase mechanism which is dependent on protein syn-

thesis (3). *N*-Acetyltransferase seems to regulate the synthesis of melatonin by limiting the availability of *N*-acetylserotonin for *O*-methylation (3).

We now report that the activity of *N*-acetyltransferase in the rat pineal gland increases rapidly at night to values which are more than 15 times greater than the day values. This circadian rhythm (4) is 180° out of phase with the rhythm in pineal serotonin (5, 6) and is similar to the rhythm in

pression of this rhythm by light seems to be mediated by an ocular route, because the removal of the eyes permits a rhythm to persist (Table 1).

A comparison of the characteristics of the circadian rhythms in pineal serotonin (5, 6) and *N*-acetyltransferase indicates that a remarkable reciprocal relation persists under normal and experimental conditions. Like the *N*-acetyltransferase rhythm, the most dramatic change in serotonin concentrations, a sharp decrease, occurs immediately after the lights are turned off (5). The serotonin rhythm is also suppressed by constant lighting, which results in continually high values, and a rhythm in pineal serotonin will persist in constant darkness and in blinded animals (6).

A circadian rhythm in norepinephrine storage in the pineal has been reported (8, 9). In contrast to the other rhythms, there is no rhythm in norepinephrine storage in constant darkness (8). The highest concentration of norepinephrine occurs at the end of the dark period (8).

Although there is poor correlation between the rhythm in the storage of norepinephrine and other pineal rhythms, other evidence indicates that the circadian rhythms in *N*-acetyltransferase and serotonin are regulated through the release of norepinephrine functioning as a neurotransmitter. Norepinephrine is found in the pineal gland in the terminals of nerve processes (11) whose cell bodies are in the superior cervical ganglia (12). In adult rats bilateral superior cervical ganglionectomy depletes norepinephrine in the pineal (13) and blocks the circadian rhythm in serotonin (6, 14). Blockage of norepinephrine synthesis by administration of the tyrosine hydroxylase inhibitor, α -methyl-*p*-tyrosine, increases the concentration of pineal serotonin (15). Likewise, administration of norepinephrine or a precursor of norepineph-

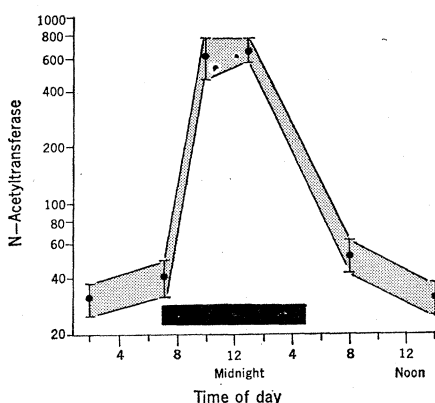


Fig. 1. The circadian rhythm in rat pineal *N*-acetyltransferase. Each point represents the mean \pm standard error of pineal *N*-acetyltransferase activities of four rats. The lighting cycle (light 14 hours: dark 10 hours) is automatically regulated. The black bar represents the dark period. Enzyme activity is given as the number of picomoles of [14 C]serotonin *N*-acetylated per gland homogenate per hour.

rine decreases the concentration of serotonin (15). Our studies in vitro directly demonstrate that norepinephrine can regulate *N*-acetyltransferase activity and causes an increased rate of acetylation of serotonin by the intact gland (3).

We tested whether serotonin could also stimulate *N*-acetyltransferase. Pineal glands obtained at 11:00 a.m. were incubated in organ culture for 21 hours under control conditions (3) and were then transferred to fresh mediums, some of which contained either serotonin ($10^{-3}M$) or norepinephrine ($10^{-4}M$). After the glands were treated with serotonin for 3 hours, no marked stimulation or inhibition of *N*-acetyltransferase was observed as compared to controls (12.5 ± 5.1 versus 6.0 ± 2.0 units). In contrast, a similar brief treatment with norepinephrine caused a greater than 15-fold increase in enzyme activity (203 ± 63 units). In similar experi-

ments, norepinephrine treatment produced a maximum stimulation of *N*-acetyltransferase at 10^{-6} mole/liter. At 10^{-4} mole/liter, tryptamine, hydroxytryptophan, histamine, tyramine, putrescine, and ethanolamine were ineffective; epinephrine, dihydroxyphenylamine, and octopamine were about as effective as norepinephrine in stimulating *N*-acetyltransferase activity (10).

The stimulation of *N*-acetyltransferase and adenylyl cyclase (16) in the pineal gland appears to require the same biogenic amines. This similarity indicates that a highly specific receptor must be associated with the adenylyl cyclase which mediates the effects of norepinephrine on *N*-acetyltransferase activity.

The circadian rhythm of serotonin and *N*-acetyltransferase under normal conditions can be explained by the following hypothesis. The initial event that synchronizes the rhythm, the "Zeitgeber," is a dark-induced release of norepinephrine from nerve endings. Norepinephrine stimulates adenylyl cyclase in the pinealocytes (16), resulting in the increased production of adenosine 3',5'-monophosphate which stimulates *N*-acetyltransferase (3). Any or all of these preliminary steps could be inhibited by light acting through an ocular photoreceptor mechanism. A rapid increase in *N*-acetyltransferase at night produces a rapid decrease in the concentration of serotonin and results in the increased concentration of *N*-acetylserotonin and melatonin (3). Pineal serotonin probably decreases because serotonin production cannot increase sufficiently to keep pace with the increased rate of acetylation. This seems possible since the activity of tryptophan hydroxylase, which is apparently the rate-limiting enzyme in serotonin synthesis in the pineal gland (6, 17) is normally about as low as daytime *N*-acetyltransferase activity. A diurnal rhythm in tryptophan hydroxylase has not been reported.

In the absence of the signals due to lighting shifts, as in blinded animals and animals maintained in darkness, other mechanisms must regulate the rhythm in *N*-acetyltransferase. Perhaps norepinephrine is released continually, but the effectiveness of this compound is periodically blocked causing the activity of *N*-acetyltransferase to oscillate.

N-Acetyltransferase activity probably regulates the amount of melatonin synthesized in vivo. Recent investigations (18) of the activity of hydroxyindole-

Table 1. *N*-Acetyltransferase activity in pineal glands. Activity is expressed as number of picomoles of [14 C]serotonin *N*-acetylated per gland homogenate per hour. Data are given as the mean \pm the standard error. The numbers in parentheses indicate the size of each group. The treatment period was 6 days. Normally, the dark period starts at 7:00 p.m. and ends at 5:00 a.m. Bilateral enucleation was performed while the animals were under light ether anesthesia.

Lighting schedule (light: dark)	Prior treatment	<i>N</i> -Acetyltransferase activity	
		11:00 a.m.	11:00 p.m.
14 : 10	None	48 \pm 6.4 (4)	1309 \pm 169 (4)
0 : 24	None	142 \pm 97 (7)	1001 \pm 245 (7)
24 : 0	None	55 \pm 15.4 (4)	53 \pm 4.6 (4)
24 : 0	Bilateral enucleation	41 \pm 5.6 (4)	1514 \pm 21 (4)

O-methyl transferase, with optimum concentration of *S*-adenosyl methionine, the methyl donor, indicate that the daytime activity of this enzyme is several times higher than the daytime activity of pineal *N*-acetyltransferase. On the basis of these measurements, it seems that *N*-acetyltransferase is the rate-limiting enzyme in melatonin synthesis during the day. At night, when *N*-acetyltransferase activity increases 10- to 30-fold, it seems probable that the rate of production of *N*-acetylserotonin exceeds the maximum possible rate of *O*-methylation. Although a small diurnal rhythm in hydroxyindole-*O*-methyl transferase has been observed (19), the highest activity reported (18) does not exceed the activity of *N*-acetyltransferase at night. This situation might result in the accumulation of *N*-acetylserotonin followed by a gradual conversion to melatonin. Hydroxyindole-*O*-methyl transferase would regulate the rate of production of melatonin under these conditions. The amount of melatonin produced during one 24-hour period, however, would be limited by the amount of *N*-acetylserotonin synthesized by *N*-acetyltransferase.

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antimicrobial systems raises the following questions. Do all the systems described function in the intact cell? Does one antimicrobial system predominate, and, if so, does this system vary with the microorganisms or the functional state of the leukocyte? Do the leukocytes have an overkill capacity and thus a reserve against the decrease or loss of one or other of the antimicrobial systems? Do intrinsic control mechanisms provide a means for increasing the efficiency of one antimicrobial system when a second system is defective? Although definitive answers to these questions cannot be given at the present time, an evaluation of the contribution of myeloperoxidase-mediated antimicrobial systems is possible because of the availability of a patient with a genetic absence of myeloperoxidase (12) and of inhibitors of peroxidase-catalyzed reactions such as azide or cyanide.

Heparinized blood was obtained from normal volunteers, from two male patients with chronic granulomatous disease, and from a patient with a genetic absence of myeloperoxidase (13). The organisms were grown, washed, and incubated with intact leukocytes or with the isolated myeloperoxidase-mediated system, and the viable cell count and extent of phagocytosis was estimated (9, 14).

Azide has a marked inhibitory effect on the killing of *Lactobacillus acidophilus* by normal leukocytes (Table 1). Comparable results were obtained with *Staphylococcus albus* and *Candida tropicalis*. Cyanide also was inhibitory although less so than azide. An inhibition of the fungicidal activity of leukocytes by cyanide has been reported (15). Azide and cyanide did not inhibit phagocytosis, and they were not microbicidal under the conditions employed. Azide and cyanide form complexes with the iron of heme-containing enzymes and, as a result, enzyme activity is lost. Myeloperoxidase is a hemoprotein, and the inhibitory effect of azide and cyanide on the microbicidal activity of myeloperoxidase and iodide ions at pH 5.0 is shown in Table 1. The addition of H₂O₂ was not required since *L. acidophilus* is a H₂O₂-producing organism. Azide was a more effective inhibitor than cyanide in the cell-free system, as was observed with intact cells.

The leukocytes of patients with chronic granulomatous disease have an impaired ability to kill certain microbial species (16), whereas other organisms

Myeloperoxidase: Contribution to the Microbicidal Activity of Intact Leukocytes

Abstract. Azide and, to a lesser extent, cyanide inhibit the microbicidal activity of myeloperoxidase and of intact normal leukocytes, but they have little or no effect on peroxidase-negative leukocytes. The contribution of the azide-sensitive (peroxidase-dependent?) systems to the total microbicidal activity of normal leukocytes is considerable. The azide-insensitive antimicrobial systems are more highly developed in peroxidase-negative leukocytes than in normal leukocytes, thus suggesting an adaptation.

The importance of intact intraleukocytic antimicrobial systems in the host defense against invading microorganisms is emphasized by the marked increase in susceptibility to infection in patients with severe neutropenia or with functionally abnormal granulocytes (such as in chronic granulomatous disease of childhood). After phagocytosis, rupture of the leukocyte granules occurs with the release of their contents into the phagocytic vacuole (1).

Among the granular contents are lysozyme (2, 3), cationic proteins (4), and myeloperoxidase (3, 5); all of these have antimicrobial properties. There also is a fall in intravacuolar pH (6) and the production of H₂O₂ by the cell (7). The antimicrobial effect of H₂O₂ is increased considerably by myeloperoxidase and an oxidizable cofactor such as iodide, bromide, chloride, or thiocyanate ions (8-11).

The complex nature of the leukocytic